STUDIES IN BLOOD PRESERVATION*

Fate of Cellular Elements and Prothrombin in Citrated Blood

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IN A previous article[2] we reported the changes observed in blood preserved with heparin. The results presented in this study are typical of a large number of observations on citrated blood.

METHOD

Five cubic centimeters of freely flowing venous blood were collected in each of thirty-five sterile flat-bottomed tubes containing 0.5 c.c. of 3.5 per cent sodium citrate solution as the anticoagulant. The blood was kept in a refrigerator at a temperature of from 4° to 6° C. throughout the period of the experiment.

Each day one tube was taken from the refrigerator and the following determinations were made: red blood cell count, hemoglobin, white blood cell count, differential white blood cell count, platelet count, and fragility test.

The plasma clotting time was done on two samples of 50 c.c. of blood by the method of Quick,[7, 8] who postulates that the rate of coagulation is a function of the concentration of prothrombin, and the production of thrombin in oxalated plasma is proportional to the concentration of prothrombin if an excess of thromboplastin is present and an optimal amount of calcium is added. The plasma was recalcified with calcium chloride at a constant temperature of 40° C. in the presence of human brain tissue emulsion to supply the thromboplastin substance, the end point being recorded by the shift on a photoelectric cell galvanometer.

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RESULTS

Donor, W. T. S.

COMPARATIVE VALUES OF BLOOD IN HEPARIN AND IN SODIUM CITRATE

	HEPARIN	SODIUM CITRATE 0.35 PER CENT	
Hematocrit (3)	48.5	42.3	per cent cells
Plasma specific gravity (3)	1.0249	1.0238	
Plasma proteins (3)	6.12	5.75	Gm. per cent
Whole blood potassium (10)	192.0	183.0	mg. per cent
Plasma potassium (10)	17.5	17.2	mg. per cent
Cell potassium (calculated) (10)	377.0	409.0	mg. per cent

VALUES ON INITIAL SAMPLE CORRECTED FOR DILUTION IN CITRATE

Red blood cell count	5,100,000.0	
Hemoglobin	15.6	Gm. per cent
White blood cell count	7,700.0	
Differential white blood cell count		
Polymorphonuclear leucocytes	61.0	per cent
Lymphocytes	29.0	per cent
Monocytes	7.0	per cent
Eosinophilic leucocytes	2.5	per cent
Basophilic leucocytes	0.5	per cent
Platelets	206,800.0	

The results of the counts and hemoglobin determinations are graphically represented in Figs. 1, 2, and 3.

Fragility of Erythrocytes.—The end points were poor throughout these studies. An actual curve of fragility could not be constructed on a daily basis. As late as the fifteenth day cells could be suspended in 0.45 per cent sodium chloride without complete hemolysis. Even on the thirtieth day cells carefully handled did not lake completely in 0.52 per cent sodium chloride.

Spontaneous hemolysis was first noted on the seventeenth day. Slight shaking in physiologic saline after the tenth day caused hemolysis. The cells on the tenth day are slightly less resistant than those on the first, and those stored for thirty days are definitely more fragile than those stored for ten days.

Prothrombin.—In the first series plasma clotting times were run for a period of fifty-two consecutive hours at one-hour (toward the end, two-hour) intervals. This was done with the aid of Dr. Kenneth Olsen. There was a rapid rise in clotting times in the first fifteen hours; at the end of this period the prothrombin content had been reduced to ineffectual levels. The curve was similar to that later published by Rhoads and Panzer[9] and seemed to indicate that bloods stored for periods longer than a day would be ineffectual in treating hemorrhage which resulted from a deficiency of prothrombin.

Eight-day-old blood, however, was given a jaundiced patient with a hemorrhagic tendency, and bleeding stopped. This was reported to Dr. Olsen who made up a new extract of rabbit brain and repeated these tests on blood supplied to him from the blood bank. To his surprise this time there was no sudden loss of prothrombin concentration.

Through the courtesy of Dr. Grant Sanger we have been able to obtain the observations tabulated in Table I on separate bloods, each time being very careful to use fresh brain extract.

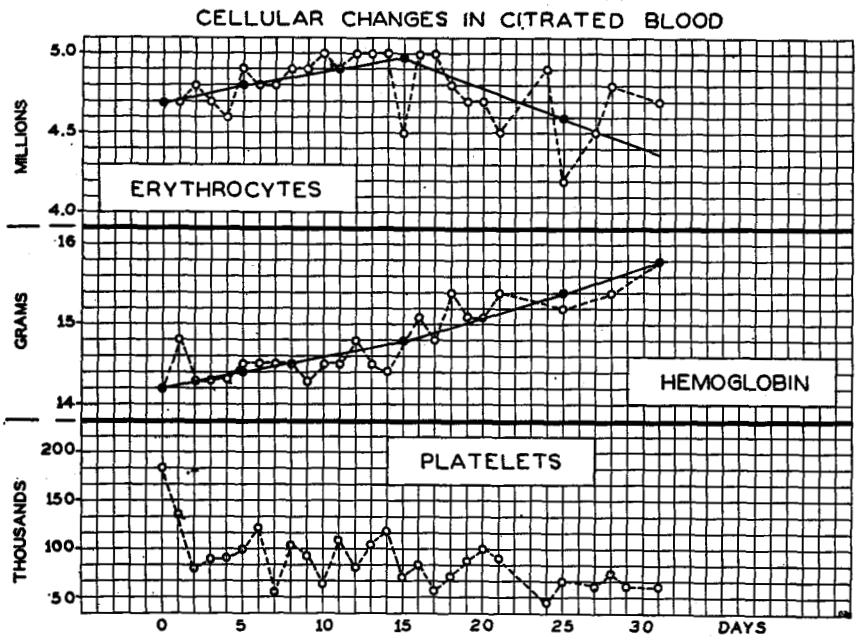

Fig. 1.—Red blood cell counts, corrected for dilution, varied between 5.5 and 4.6, the mean being 5.1 millions. Here there is an actual loss of 1,000,000 to 1,500,000 cells at the end of thirty days. Hemoglobin values varied between 15.6 and 16.3 Gm. per cent. The gradual rise is attributed to evaporation. Platelets fell from 206,800 to 87,800 in forty-eight hours, then remained constant for about fifteen days, after which time counts were difficult.

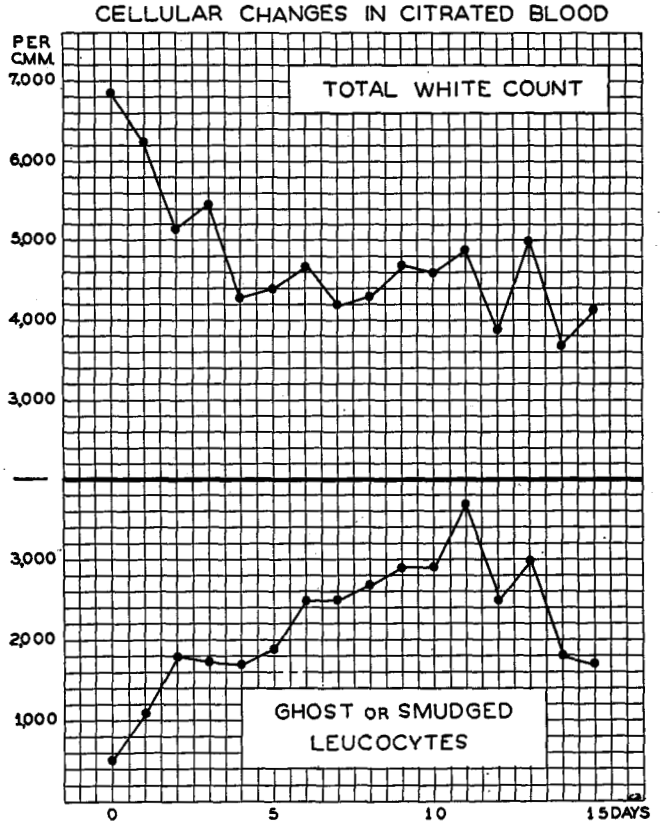

Fig. 2.—The total white blood cell count as observed in the chamber fell 27 per cent in the first five days. Careful differentiation of cells in the chamber and observation on a slide showed that 75 per cent of the total had no nuclei or were so fragile that they broke and left only a smudge by the twelfth day.

DISCUSSION

Red Blood Cells.—The maintenance of total erythrocyte counts at an approximately constant level in heparinized blood[2] and the moderate destruction of erythrocytes in citrated blood after the fifteenth day of storage suggest that cell destruction plays, at most, only a small part in the steady increase of the potassium content of the plasma,[11] and insures the recipient of receiving a large percentage of functioning cells after periods of storage of at least a month.

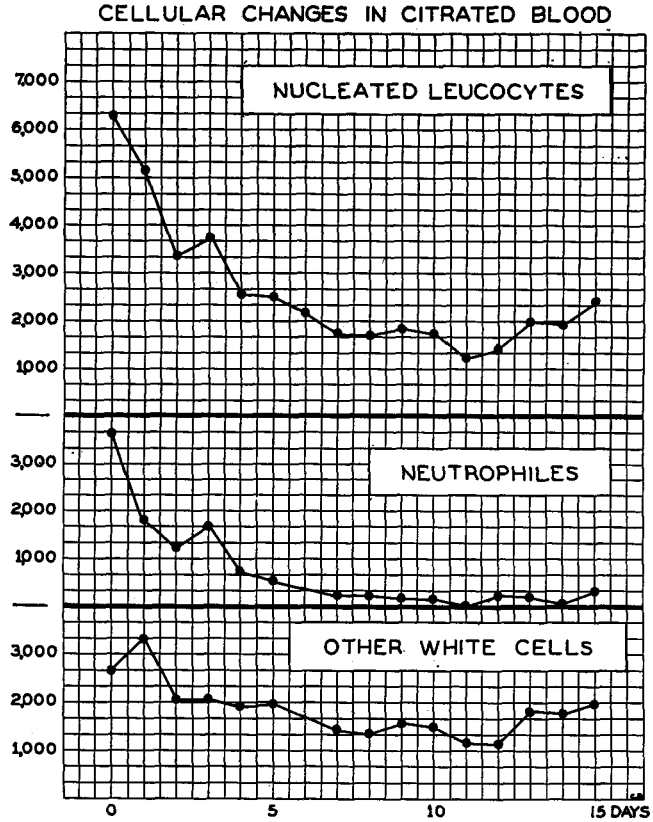

Fig. 3.—The nucleated leucocytes, presumably the only ones capable of function, decreased nearly 50 per cent in the first three days. The polymorphonuclear leucocytes diminished 50 per cent in twenty-four hours, accounting almost completely for the drop in total count. By the sixth day it was difficult to be sure that any remained. Eosinophiles were well preserved. Lymphocytes remained almost constant. Monocytes were difficult to differentiate.

Hemoglobin.—The hemoglobin content of the whole blood remains constant, though at the end of thirty days 20 per cent of it may be found in the plasma.

White Blood Cells.—The polymorphonuclear leucocytes may show swelling, hazy cytoplasm, and poorly staining nuclear granules as early as twenty-four hours after storage. Disintegration is extremely rapid and may be responsible for the steep rise of the potassium curve in the first week;[11] likewise it may play a part in decreasing bactericidal properties.[4, 5]

The lymphocytes are more resistant. At the end of thirty days they are easy to recognize when seen.

The eosinophiles are most resistant, the eosinophilic granules remaining particularly bright even when the nuclear material has faded or broken up.

Platelets.—Platelets disintegrate more rapidly in heparinized blood[2] than in citrated blood. In both bloods they reach their low point in about three to five days. Platelets remaining stay fairly fixed until clumping makes counting difficult about the fifteenth day.

TABLE I

PROTHROMBIN CONCENTRATIONS IN PLASMA OF STORED BLOOD

DATE BLOOD STORED	DATE TEST MADE	AGE IN DAYS	PROTHROMBIN CONCENTRATION (PER CENT)
10/27/39	2/21/40	117	47
10/27/39	2/21/40	117	38
11/20/39	2/21/40	94	41
12/15/39	2/21/40	69	49
1/31/40	2/21/40	21	52
2/28/40	3/12/40	13	100
3/ 1/40	3/12/40	11	100
2/16/40	2/26/40	10	68
2/23/40	3/ 1/40	8	84
2/23/40	3/ 1/40	8	84
2/26/40	3/ 5/40	8	60
3/ 4/40	3/12/40	8	100
3/ 4/40	3/12/40	8	80
3/ 4/40	3/12/40	8	100
2/19/40	2/26/40	7	100
2/19/40	2/26/40	7	76
2/23/40	2/29/40	6	100
2/23/40	2/29/40	6	100
2/16/40	2/21/40	5	74

Prothrombin.—The results using the Quick method of determining the prothrombin concentration yield values similar to those reported by Lord and Pastore[6] using the Brinkhous, Smith, and Warner method.[1] This suggests that the use of brain extract, which has been kept too long, may account for the discrepancy between reported findings. Dr. Olsen deserves credit for making this very essential observation.

SUMMARY

1. In citrated blood there is some loss in the number of red blood cells beginning about the fifteenth day and amounting to from 1,000,000 to 1,500,000 cells by the end of the month.

2. The hemoglobin content remains constant in the total sample, although 15 to 25 per cent may diffuse out of the cells into the plasma in one month.

3. The polymorphonuclear leucocytes are diminished to 50 per cent in forty-eight hours and are amorphous masses in fifteen days.

4. The lymphocytes and eosinophiles do not disintegrate so rapidly; the latter are particularly well preserved. The monocytes are difficult to trace.

5. The platelets fall rapidly to a level ranging from 50,000 to 80,000 and remain at this level for about fifteen days, at which time counts become difficult to make.

6. The fragility of red blood cells slowly increases with increasing age; exact curves are difficult to establish.

7. The prothrombin level is maintained above 40 per cent of normal concentration for a period of at least four months. The use of old brain extract

will cause clotting times which are too rapid, thereby giving a false picture of the true degree of efficacy of preserved blood in the therapy of hemorrhagic diseases associated with low prothrombin concentrations.

We are indebted to Miss Hildegard Menzel for aid in the prothrombin determinations and to Mr. Josiah Lasell for many hematologic studies.

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